



Application No. 09/779,334, Amendment Dated June 14, 2004, Replacement Sheet

Subtilisin Structure-Function Correlation

Thermostablility Motifs

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Subtilisin Structure—Function Correlation

W A A A W W H I A W W S L G S D B	Thermostablility Motifs APSTFLERAVNYATSQGVLVIAATGNNGSGSVGYPARYANANAV
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NAAANNHIANNSLGSDAPSTILE	RAUNYATS Q G V L V I A A T G N N G S G S V G Y P A R Y A N A N A V
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WAAANNMHIANNS LGSDAPSTTL	E R A V N Y A T S Q G V L V I A A T G N N G S G S V G Y P A R Y A N A N A V
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WAAANNHIANNSLGSDAPSTTL	E R A V N Y A T S Q G V L V I A A T G N N G S G S V G Y P A R Y A N A M A V
WAATNNMHIANNSLGSDAPSTIL	E R A V N Y A T S Q G V L V I A A T G N M G S G S V G Y P A R Y A N A M A V
H A A A N N M H I A N N S L G S D A P S T T L	ERAVNYAT SQ'GVLVIAATGNNGSGSVGYPRRYANAV
W A A A N N H I A N N S L G S D A P S T P L	ERAVNYA T S Q G V L V I A A T G N N G S G S V G Y P A R Y A N A M A V
HAAANN HIANNS LGSDAPSTTL	ERAVNYAT S Q G V L V I A A T G N N G S G S V G Y P A R Y A N A N A 'V
W A A A N N H I A N N S L G S D A P S 1 T L	ERAVNYATS O GVLVIAATCNNGS GSVGYPARYANANAV
WAAANNMHIANNS LGSDAPSTTL	ERAVNYATSRDVLVIAATGNNGSGSVGYPARYANAN

-162B

HAGNNGMHVANLSLGSPSPSPSPTLEQAVNSATSRGVLVVAASGAGSISYPARYANAVGATD 388

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Correlation
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Structure-Fu
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Thermostablility Motifs

Majority	Q N N R R A N F S Q Y G T G I	VNVDIVAPGVNV	OSTYPGNRYAS	LNGTSMATPHVA	OAALVK AVIAA D		
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pac. gdg	ONNRRANFS OYGTG	IDIVAPGVNV	OSTYPGNRY [V]S	MSGTSHATPHVA	GAALVK		523
4c6.seg	Q N N R R A N F S Q Y G T G I	VNVBPGVNV	OSTYPGGQYAE	LSGTSMASPHVA	GAAALVR		573
3b3.seq	QNNRRANFSQYGTG	VNVBGVNV	OSTYPGNRYAS	LSGTSHATPHVA	GAAALVK		523
je2.seq	Q N N R R A N F S Q Y G T G I	VNVPGVNV	OSTYPGNRYAS	LSGTSMATPHVA	GAAALVK		523
sh9.seq	Q N N R R A N F S Q Y G T G I	VNVSGVNV	OSTYPGNRYAS	LNGTSMATPHVA	GVAALVK		523
3a7.seq	ONNRRANFS OYGTG 1	VNVBGVNV	Q S T Y P G N R Y A S	LNGTSMATPHVA	GAALVK		523
Sb11.seq	ONNRRANFS OYGTG	VNVDIVAPGVNV	Q S T Y P G N R Y V	MNGTSHATPHVA	GAAALVK		523
4d10.seq	Q N N R R A N F S Q Y G T G I	IDIVAPGVNV	Q S T Y P G N R Y V S	MNGTSMATPHVA	GAAALVK		523
1f6.seq	Q N N R R A N F S Q Y G T.G I	IDIVAPGVNV	QSTYPGNRYVS	MNGTSMATPHVA	GVAALVK		523
4c2.seq	QNNRRANFSQYGTGI	IDIVAPGVNV	QSTYPGNRY [V]S	MNGTSMATPHVA	GVAALVK		523
	Q N N R R A N F S Q Y G T G I	VNVBGVNV	Q S T Y P G N R Y A S	LSGTSMATPHVA	GVAALVK		573

FIG 2C

Q N N N R A S F S Q Y G A G L D I V A P G V N V Q S T Y P G S T Y A SL N G T S M A T P H V A G A AAL V K Q K N P S H S N V X

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Subtilisin Structure—Function Correlation pH Shifting Motifs

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FIG 2D

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Subtilisin Structure—Function Correlation pH Shifting Motifs

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Savinase seq NAGNNGMHVANLS LGSPSPSPSPTLEQAVNS ATSRGVLVVAASGNSGAGS ISYPARYANAVGATD 188



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Subtilisin Structure—Function Correlation pH Shifting Motifs

Majority	QNNNRASFSQYGAGLD	IVAPGVGVQS1	TYPGNRYASLNG	TSMATPHVAG	VAALVKQKNPS	W S N·V X
•	140	150	160	170	180	190
5el seq	QNNNRASFSQYGAGLD	IVAPGVGVQST	TYPGNRYASLNG	TSMATPHVAG	AAALVK	523
6a4 seq	QNNNRASFSQYGAGLD	IVAPGVGVQST	TYPGSTYASLNG	TSMATPHVAG	VAALVK	523
9b4 seq	QNNNRASFSQYGAGLD	IVAPGVGVQST	TYPGSTYASLNG	TSMATPHVAG	VAALVK	523
lc10 seq	QNNNRASSQYGAGLD	IVAPGVGVQS7	TYPGNRYASLNG	TSMATPHVAG	VAALVK	523
7a2 seq	ANFSQYGTGI	IVAPGVEIES	TYPGSSYDSLRG	TSMATPHVAG	AAALVK	523
4d7 seq	QNNRRANFSQYGIGID	IVAPGVNVQS	TYPGNRYASLNG	TSMATPHVAG	VAALVK	523
eb6 seq	QNNNRASFSQYGAGLD	IVAPGVNVQS	TYPGSTYDSLSG	TSMATPHVAG	VAALVK	523
eg6 seq	QNNRRANFSQYGTGID	IVAPGVNVQST	TYPGGQYAELSG	TSMASPHVAG	AAALVK	523
	QNNRRANFSQYGTGID	IVAPGVNVQS7	TYPGNRYASLNG	TSMATPHVAG	VAALVK	523
6b11 seq	QNNRRANFSQYGID	IVAPGVNVQST	TYPGNRYASLNG	TSMATPHVAG	VAALVK	523

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Subtilisin Structure—Function Correlation Activity in DMF Motifs

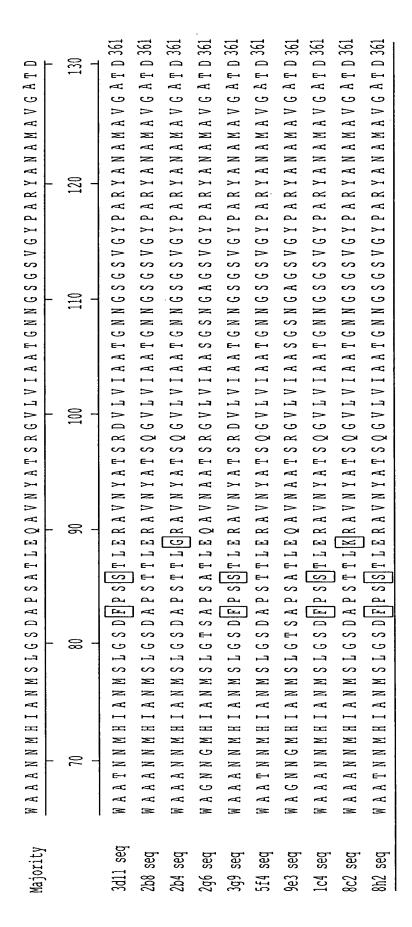
GASFVPGE	PSTODGNGHG	THVAGTIAA	LNNSIGVLGVA	PNADLYAVKVL	GANGSGSVSG	IAQGLE
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GASFVPGEPSTQDGNGHGTHVAGTIAALNNSIGVLGVAPSAELYAVKVLGASGSGSVSSIAQGLE193 Savinase seq



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Subtilisin Structure—Function Correlation Activity in DMF Motifs



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Savinase seq

FIG 2H

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Subtilisin Structure—Function Correlation Activity in DMF Motifs

Majoritv	QNNRRANFSQYGTGIDI	VAPGVNVQSTY	PGNRYASLNG	TSMATPHVAG	VAALVKQKNPSW	SNVK
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	140	150	160	170	180 190	
3d11 seq	QNNRRANFSQYGTGIDI	VAPGVNVQSTY	PGNRYASING	TSMATPHVAG	AAALVK	523
2b8 seq	QNNRRANFSQYGTGIDI	VAPGVNVQSTYI	PGNRYASLNG	TSMATPHVAG	VAALVK	523
2b4 seq	QNNRRANFSQYGTGIDI	VAPGVNVQSTYI	PGNRYASING	TSMATPHVAG	VAALVK	523
2g6 seq	QNNRRANFSQYGTGIDI	VAPGVNVQSTYI	PGNRYVSMNG	TSMATPHVAG	VAALVK	523
3g9 seg	QNNRRANFSQYGTGIDI	VAPGVNVQSTYI	PGNRYASLNG	TSMATPHVAG	VAALVK	523
	QNNRRANFSQYGTGIDI	VAPGVNVQSTYI	PGNRYARLNG	TSMATPHVAG	VAALVK	523
9e3 seq	QNNRRANFSQYGTGIDI	VAPGVNVQSTYI	PGNRYVSMNG	TSMATPHVAG	VAALVK	523
1c4 seq	QNNRRANFSQYGTGIDI	VAPGVNVQSTYI	PGNRYASING	TSMATPHVAG	AAALVK	523
8c2 seq	QNNRRANFSQYGTGIDI	VAPGVNVQSTYI	PGNRYASLNG	TSMATPHVAG	AAALVK	523
8h2 seq	QNNRRANFSQYGTGIDI	VAPGVNVQSTYI	PGNRYASLNG	TSMATPHVAG	VAALVK	523

QNNNRASFSQYGAGLDIVAPGVNVQSTYPGSTYA SLNGTSMATPHVAGAAALVKQKNPSWSNVK 590

Savinase seg